MEMBRANE-BOUNDED NUCLEAR BODIES IN A DIVERSE RANGE OF MICROBIAL SYMBIONTS OF GREAT BARRIER REEF SPONGES

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Thin sections of chemically fixed tissue of several sponge species collected from Heron Island, Great Barrier Reef, including *Jaspis stellifera, Pseudoceratina crassa* and *Axinyssa* sp., were examined to investigate the cell organisation of bacteria-like microbial symbionts present. Such symbionts have been observed in these sponges to occur as a diverse range of morphotypes based on cell shape and cell wall type. A variety of different symbiont morphotypes were found to possess a membrane-bounded nucleoid, a feature not expected in prokaryotes. These had been previously observed by us in one symbiont morphotype in the Micronesian coralline sponges *Stromatospongia micronesica* and *Astrosclera willeyana.* Several distinct microbial morphotypes containing membrane-bounded nuclear bodies were observed in Great Barrier Reef sponges, only one of which resembled the type which we have previously observed in the two Micronesian sponges. In all these forms, the fibrillar nucleoid was surrounded by a single bilayer membrane, in most morphotypes defining a compartment also containing electron-dense particles resembling ribosomes or other nucleoplasmic pre-ribosomal material; such material was sometimes less dense and sometimes more dense than the cytoplasmic particulate material. Cell wall structure of the morphotypes broadly included both Gram-negative, outer membrane-bounded types, as well as a clear subunit S-layer type structure resembling that of known Archaea including crenarcheotes. Cytoplasmic membranes can be clearly seen in some cases as distinct from nuclear body membranes, excluding plasmolysis as an explanation for membrane-boundedness of nuclear bodies. The phylogenetic relationships of these microbes may be diverse if reflecting wall type, but at least some appear to be most likely to represent members of the Domain Archaea, perhaps resembling the crenarcheote *Cenarchaeum symbiosum* described from North American *Axinella* sp. ❐ *Porifera, Bacteria, Archaea, nucleoids, membrane-bounded, sponge symbionts, electron microscopy, ultrastructure.*

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There are 2 known major types of cell organisation, prokaryotic where the DNA of the genome is free in the cytoplasm and not confined to a special compartment, and the eukaryotic, where the genomic DNA is confined to a double membrane-bounded organelle, the nucleus, and in addition any other of several double-membrane-bounded organelles such as mitochondria and chloroplasts may be present (but not in all eukaryotes, e.g., archezoan protozoa such as *Giardia*). In the prokaryote the naked genomic DNA in chemically fixed cells often appears to be folded or condensed into a fibrillar structure and this ultrastructural entity is termed the '*nucleoid*'. The prokaryotic form is characteristic of most known species within two of the three great Domains of life defined by contemporary molecular systematics, the Bacteria and the Archaea, while the eukaryotic form is known so far only within the Domain Eucarya and not in the other two Domains (Woese et al., 1990). Several questions about such a classification of cell organisation can be posed, however. Are these the *only* forms of cell organisation which have evolved, or might there not be intermediate forms or even more complex ones, hitherto undiscovered due to our limited knowledge of biodiversity?

Related to this is a second question- are membrane-bounded nuclei or their analogues

exclusive to the Domain Eucarya, or might they or some analogous form of organelle occur in those two Domains of life thought to harbour only prokaryotic cells?

The first indication that there might be alternative forms of cell organisation to those classical known ones was discovered in a distinct division or phylum of the Bacteria, the planctomycetes (Order *Planctomycetales*), where one species, *Gemmata obscuriglobus*, possesses a genome bounded by two membranes (Fuerst & Webb, 1991) while in another two, *Pirellula marina* and *Pirellula staleyi*, a single membrane separates the compartment containing the genomic DNA from the rest of the cell (Lindsay et al., 1997). We present here evidence that several distinct morphotypes of sponge symbionts (only one of which has been described by us previously; see Fuerst et al., 1998), reveal further examples of structurally novel types of cell organisation in which the genomic DNA appears compartmentalised by a single membrane from the rest of the cell cytoplasm, and that these may occur in microorganisms resembling members of the Domain Archaea, and present new data to support these findings.

MATERIALS AND METHODS

Stromatospongia micronesica and *Astrosclera willeyana* were collected from Guam (Micronesia), and *Pseudoceratina crassa*, *Jaspis stellifera* and *Axinyssa* sp were collected from Heron Island (Great Barrier Reef), at sites previously described in detail (Fuerst et al., 1998). Sponge tissue samples were chemically fixed with glutaraldehyde followed by osmium tetroxide before resin embedding and thin sectioning, via protocols including hydrofluoric acid treatment of either tissue blocks or Epon resin-embedded block faces, and uranyl acetate-lead citrate stained thin sections were examined via transmission electron microscopy, as described previously (Fuerst et al., 1998). For immunolabelling experiments, samples of *Jaspis stellifera* were fixed in 2% glutaraldehyde-4% paraformaldehyde fixative in 0.1M cacodylate buffer, and further processed as described in Fuerst et al. (1998). Immunolabelling of thin sections was via use of an anti-ds+ss DNA antibody (Boehringer-Mannheim) and goat anti-mouse IgM conjugated to either 10nm or 5nm colloidal gold, as described previously (Lindsay et al., 1997), and sections were then stained with uranyl acetate and lead citrate. Labelling of sections to localise RNA using an RNase-colloidal gold (10nm) conjugate was performed essentially as described in Lindsay et al. (1997), followed by staining as above. In cases where double labelling was performed, RNase gold labelling was performed first, followed by anti-DNA gold immunolabelling. Goat anti-mouse IgM conjugated to 5 nm colloidal gold was used for labelling of DNA when double-labelling experiments involving both DNA and RNA labelling were performed.

Voucher samples of *Axinyssa* sp. nov. and *Pseudoceratina crassa* are held at the Queensland Museum as QMG312575 and QMG304915 respectively (identified by Dr John Hooper).

RESULTS AND DISCUSSION

Bacteria-like symbionts of varying morphotype were common in mesohyl of the tissue of the sponge species collected from Heron Island, including *Jaspis*, *Pseudoceratina* and *Axinyssa* spp. (see Fig. 1A). These associates were found to include a diversity of morphotypes displaying a novel form of compartmentation, in which either the fibrillar nucleoid representing the prokaryote chromosomal DNA was surrounded by a single membrane, or , in one type, where an inverse of this compartmentation topology was displayed. In the latter type, a single membrane separates one organelle-like region of the cytoplasm from a second region of cytoplasm containing the nucleoid. The compartmented morphotype in which the nucleoid is bounded by a single membrane has been previously described by us in sponges from Pacific Micronesia, *Stromatospongia micronesica* and *Astrosclera willeyana* (Fuerst et al., 1998). The dramatic distinction of this morphotype from bacteria with a classical prokaryotic 'naked' fibrillar nucleoid, free in the cytoplasm, is seen in Figure 1B of the mesohyl of *S. micronesica*. This first kind of compartmented morphotype, here seen in an actively dividing cell, displays a characteristically 'butterfly' shaped nuclear body surrounded by a single membrane. The cell wall structure is of a regular subunit type consistent with membership of the Domain Archaea (see Fuerst et al., 1998, and the discussion of this wall type in other morphotypes below). This morphotype is typical of all the nucleated symbiont morphotypes, in the consistency of features such as the cell size, the absence of membrane-bounded organelles other than the nuclear body, and cell wall structure,

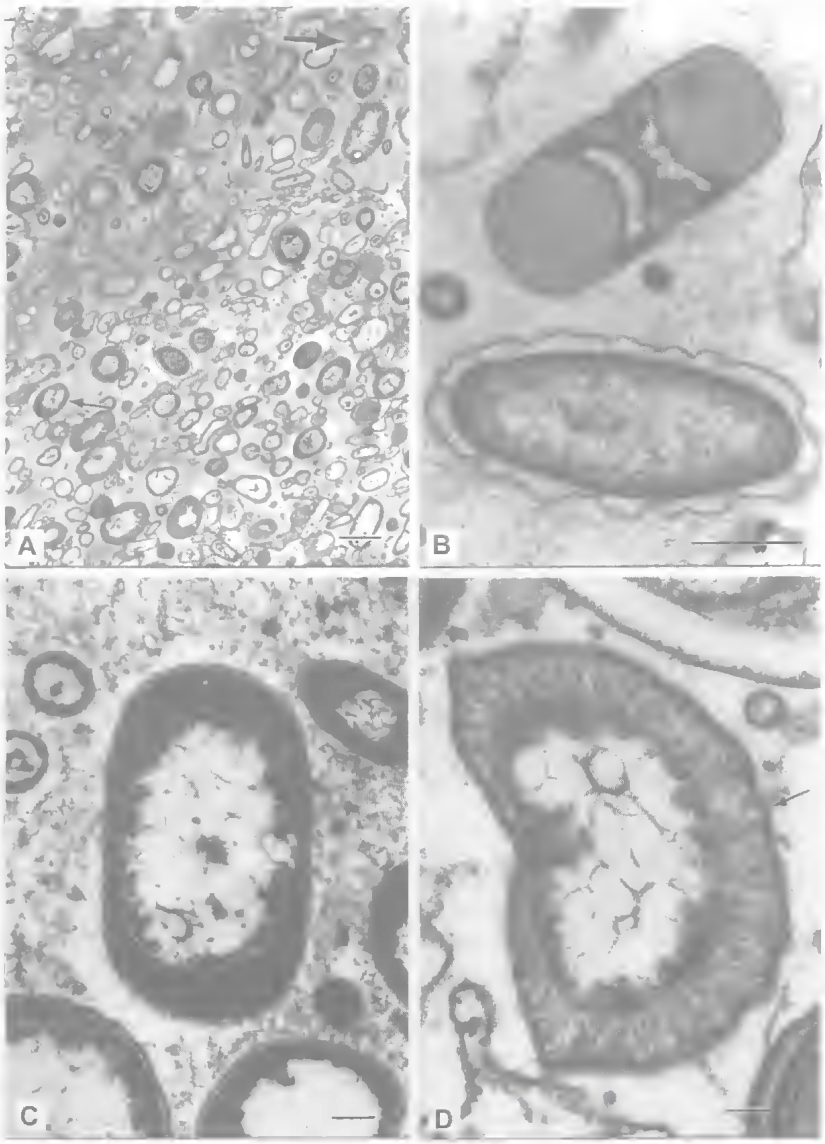

FIG. 1. A, Electron micrograph of thin section of mesohyl from sponge tissue of *Jaspis stellifera* from the Great Barrier Reef, with a diverse range of symbiont morphotypes apparent, based on a combined consideration of cell size, shape and internal structure. For example, the large arrow indicates a Morphotype 1 cell and the small arrow indicates a Morphotype 2 cell. (Scale bar 1 μm). B, Electron micrograph of thin-section of sponge tissue from *Stromatospongia micronesica* showing two symbiont morphotypes of contrasting internal structure. One morphotype (Morphotype 1 in the typing scheme used in this paper), the uppermost cell in this figure, possesses a membrane-bounded nuclear body region in a dividing cell (evidence for active viable cell growth of this type in the tissue) and the second morphotype is a cell with normal bacterial (prokaryotic) ultrastructure with fibrillar nucleoid DNA free in the cytoplasm. Note in the Morphotype 1 cell that the nuclear body in each cell half displays an outer electron dense region as well as a central fibrillar nucleoid region. (Scale bar 1 μm). C, Electron micrograph of thin-sectioned Morphotype 2 (short fat rod) symbiont from *Pseudoceratina crassa* from the Great Barrier Reef; note the membrane-bounded nuclear body and that this type has a relatively electron-dense cytoplasm external to the nuclear body compared with other morphotypes (e.g., Morphotype 1 seen in Fig. 1B). (Scale bar 200nm). D, Electron micrograph of thin-sectioned Morphotype 3 (D-shaped cell) symbiont from *Pseudoceratina crassa*. This D-shaped cell has a clear membrane-bounded nuclear region as well as radiating fibres in the cytoplasm outside the nuclear region. The cell wall displays a regular subunit periodic structure (arrow). (Scale bar 200nm).

with most probable phylogenetic relationships of these symbionts to non-eukaryote organisms such as Bacteria or the Archaea. This morphotype with 'butterfly' nuclear body has now been found also in sponges from the Great Barrier Reef, including *Jaspis stellifera*, *Pseudoceratina crassa* and *Axinyssa* sp. This morphotype has thus now been found to be distributed among at least 5 different sponge genera (*Stromatospongia, Astrosclera, Jaspis, Pseudoceratina* and *Axinyssa*) and in at least two different geographical locations in the Western Pacific. However, not only was this morphotype found in both Great Barrier Reef and Micronesian sponges, but it turns out to constitute only one of several different morphotypes which display membrane-bounded nuclear regions, or at least membrane-bounded compartments, separating the cell interior into a nucleoid- and non-nucleoid-containing region. These compartmented cell symbiont morphotypes could be distinguished from each other on the basis of the following criteria or combinations of such criteria; cell wall structure, cell shape, texture (fine structure) of the cytoplasm outside the nuclear body, and type of cell compartment (e.g. nucleoid-containing versus nucleoid-devoid, or butterfly-lobed versus round in outline). In most of these morphotypes, the nucleoid is surrounded by a single membrane separating the nuclear region from the rest of the cell, as exemplified most dramatically in the type with butterfly-shaped nuclear body found first in *S. micronesica* and *A. willeyana*. In one morphotype only, the nucleoid appears external to a central single-membrane-bounded compartment effectively separating the cell into two compartments, one with nucleoid and the other without.

In the morphotype classification used in this paper, Morphotype 1 is considered to be the type with a butterfly-shaped membrane-bounded nuclear region. The second type, referred to as Morphotype 2, is a short, fat, rod morphotype (Fig. 1C). In Morphotype 2, the nuclear region is bounded by a single membrane (Fig. 3A), but has a relatively electron-dense cytoplasm compared with Morphotype 1.

Morphotype 3 is a D-shaped cell; this type not only shows a characteristic cell shape and a very clear membrane-bounded nuclear region but also displays radiating fibres in the cytoplasm outside this region (Fig. 1D). Most interesting of all, the cell wall displays a structure of regular subunits, compatible with possible membership of the Domain Archaea (Fig. 1D). Members of the Archaea have been classically considered to include organisms inhabiting very hot hydrothermal and volcanically heated waters as well as methane-generating anaerobes and organisms growing in saturated salt (Woese et al., 1990). However, recently they have been reported from less extreme marine habitats (e.g., Atlantic, Pacific and Antarctic seawater; see DeLong, 1992, DeLong et al., 1994 and Fuhrman et al., 1992), including, very significantly for this context, a report of the cold water archaeon sponge symbiont *Cenarchaeum symbiosum* from a Californian coastal *Axinella* species, confirmed as an archaeon by gene probing (Preston et al., 1996). These Archaea all belong to the so-called marine group I cluster which is part of the Kingdom Crenarcheota within the Domain Archaea (DeLong, 1992). In addition to their occurrence in a sponge, such bacteria have also been recently found to occur in holothurian gut and marine fish gut (McInerney et al., 1995; Van Der Maarel et al., 1998).

Another morphotype (Morphotype 4) displays a cell wall with a structure consistent with Gram-negative bacteria - those known to give a negative Gram stain reaction. The wavy nature of the outer cell wall membrane is very clear, consistent with Gram-negative type cell wall; in this type the DNA fibrils occupying most of the nuclear body are very obvious and enclosed within a very distinct membrane (Fig. 2B).

Morphotype 5 is another morphotype with subunit cell wall consistent with membership of the Archaea, this time a rod without a D-shape bias (Fig. 2B-C). Note also the regular subunit 2-D lattice structure visible in the grazing section portion of wall (arrow in Fig. 2B). Morphotype 6 exhibits a characteristically blebbed cell wall membrane, containing a membrane-bounded internal body but in this case without a nucleoid - the nucleoid is in the other 'cytoplasmic' cell compartment (Fig. 2D). Thus the cell is still divided into a non-nucleoid-containing and a nucleoid-containing compartment, albeit in a reverse topological sense to that found in the other morphotypes with a membrane-bounded internal or centrally located nucleoid-containing compartment.

To summarise the symbiont morphotypes described above, there are six morphotypes occurring in Great Barrier Reef sponges *J. stellifera, P. crassa* and *Axinyssa* sp. The first five are Morphotype 1 (rods with "butterfly" nuclear

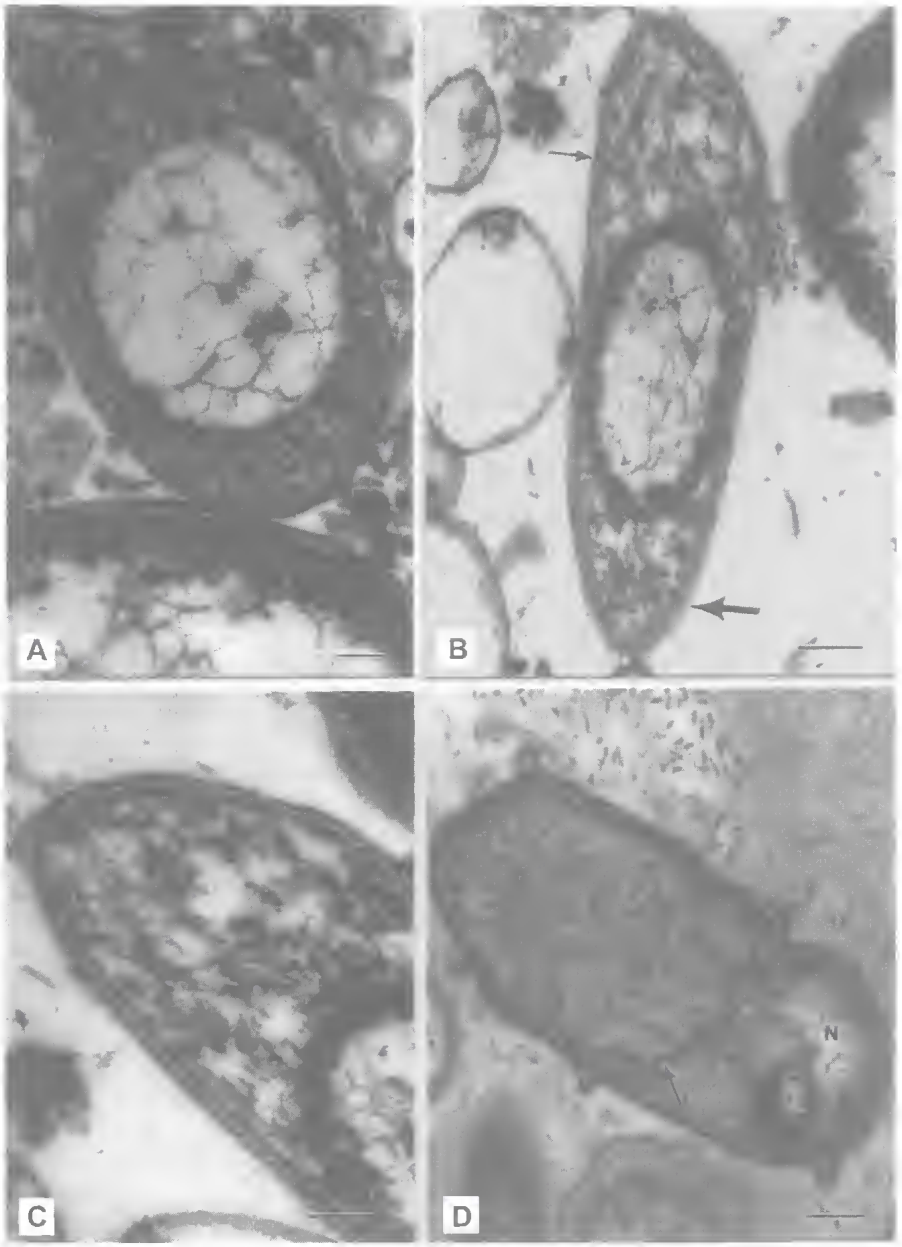

FIG. 2. A, Electron micrograph of thin-sectioned Morphotype 4 (Gram-negative walled cell) symbiont from *Pseudoceratina crassa* displaying a wavy outer cell wall membrane similar to that in walls of Gram-negative bacteria, as well as a single-membrane-bounded nuclear region with DNA fibrils occupying most of the nuclear body. (Scale bar 100nm). B, Electron micrograph of thin sectioned Morphotype 5 (regular subunit walled normal rod) symbiont from *Pseudoceratina crassa* displaying a cell wall consisting of regular subunits (small arrow), especially visible as a periodic lattice in a portion in which a grazing section has occurred (large arrow). (Scale bar 200nm). C, Enlargement of thin sectioned Morphotype 5 (regular subunit walled normal rod) symbiont from *Pseudoceratina crassa* shown in Fig. 2B displaying a cell wall consisting of regular subunits. Note also the portion of the nuclear body and clearly displayed single membrane envelope of this body towards lower right hand side of figure. (Scale bar 100nm). D, Electron micrograph of thin sectioned Morphotype 6 symbiont from *Pseudoceratina crassa* displaying a characteristic blebbed cell wall outer membrane. A membrane-bounded internal body (arrow) is present but the nucleoid (N) is situated in the cell compartment external to this inner body, in contrast to all other morphotypes described in these figures. (Scale bar 200nm).

bodies); Morphotype 2 (short fat rods with electron-dense cytoplasm); Morphotype 3 (D-shaped cells with subunit ("archaeal") walls); Morphotype 4 (rods with Gram-negative outer membrane walls); and Morphotype 5 (cells with large subunit ('archaeal') walls). All of these 5 types contain a membrane-bounded nucleoid-containing nuclear body, more or less centrally located within the cell. Note again that 'nucleoid' is here being used in the bacteriological sense of a fibrillar genomic DNA bundle, which is not normally membrane-bounded. Morphotype 6 differs from the first five. It consists of rods with blebbed cell wall membrane but with a membrane-bounded internal compartment without nucleoid (Fig. 3A-B). The cell is effectively divided into two compartments, however, in an analogous manner to the compartmentalisation in the first five morphotypes, but with a reversed topology.

It should be noted that every morphotype has been seen in all these Great Barrier Reef sponges examined (*P. crassa, J. stellifera* and *Axinyssa sp.*). Thus the diversity of morphotypes possessing membrane-bounded nuclear bodies we have seen may be a widely distributed phenomenon unrelated to host specificity.

The morphotypes described here appear to be sub-types of the types E and 4, previously described in published studies (Vacelet, 1975; Wilkinson, 1978). In those studies, the appearance of types E and 4 was explained by the occurrence in these bacteria of an unusually large periplasm, that is, the region between cell wall and cytoplasmic membrane (Vacelet, 1975; Wilkinson, 1978). In this interpretation, the membrane-bounded nuclear body we have seen would merely represent the cell protoplast (cell cytoplasm contents) surrounded by a retracted cytoplasmic membrane, and the space between nuclear body membrane and cell wall would represent a very wide 'periplasm' rather than true cytoplasm. We favour an alternative interpretation, in which the membrane bounding the nuclear region is a true internal membrane rather than representing cytoplasmic membrane, which is closely appressed to the cell wall in the symbionts we have observed and therefore often difficult to detect. Supporting this is the clear indication of cytoplasmic membrane for at least three Morphotypes as shown in Morphotypes 2 and 3 (Fig. 3A-B) and Morphotype 1 (Fig. 2c in Fuerst et al., 1998). Also consistent with this interpretation in Morphotype 1, and several other of the Morphotypes, is the uniform distribution of cytoplasm within the space between the cell wall and internal nuclear body-bounding membrane, a distribution unlikely if plasmolysis and retraction of cytoplasmic membrane were responsible for this space.

To investigate this problem further, we employed immunogold labelling methods to localise DNA and RNA within the cell and thus determine the location of true cytoplasm. Figure 3C shows a nucleated symbiont Morphotype 1 cell from *J. stellifera* in which the DNA has been localised via immunogold labelling using mouse monoclonal antibody against single-stranded and double-stranded DNA detected via goat anti-mouse antibody conjugated to 10nm colloidal gold particles. All the cell's DNA is localised exclusively within the membrane-bounded nuclear body, suggesting that this must be the location of the chromosomal, genomic DNA. Intracellular RNA was localised in this morphotype using the slightly different enzyme cytochemistry approach employing RNase conjugated directly to colloidal gold. By this method, RNA in these cells is located throughout the cytoplasmic region external to the membrane-bounded nuclear body (Fig. 3D), as well as being present to minor extent in the nuclear body, as would be expected if transcription is to occur using a genomic DNA template. This occurrence of RNA in the cytoplasm outside the membrane-bounded nuclear region supports an interpretation of symbiont ultrastructure in which the space between the nuclear body membrane and the cell periphery is occupied by true cytoplasm and is not merely an unusually large periplasm between a retracted cytoplasmic membrane and the cell wall, and in which the nuclear body is thus a true intracytoplasmic membrane-bounded compartment of the cell. Some RNA also appears in the electron-dense-particle-rich outer zone within the nuclear body itself, as would be expected if cell RNA is transcribed from DNA in the nuclear body. Double-labelling using both DNA and RNA labelling methods with differently sized gold particles confirms the distribution of DNA and RNA relative to the nuclear body found via separate use of DNA and RNA labels (Fig. 4A)

Gold labelling was also used to examine the problem posed by Morphotype 6 symbionts, where there appears to be a non-nucleoid-containing internal membrane-bounded body. A combination of RNase-gold and anti-ss and dsDNA antibody immunogold labelling demonstrated that most of the cell RNA appeared

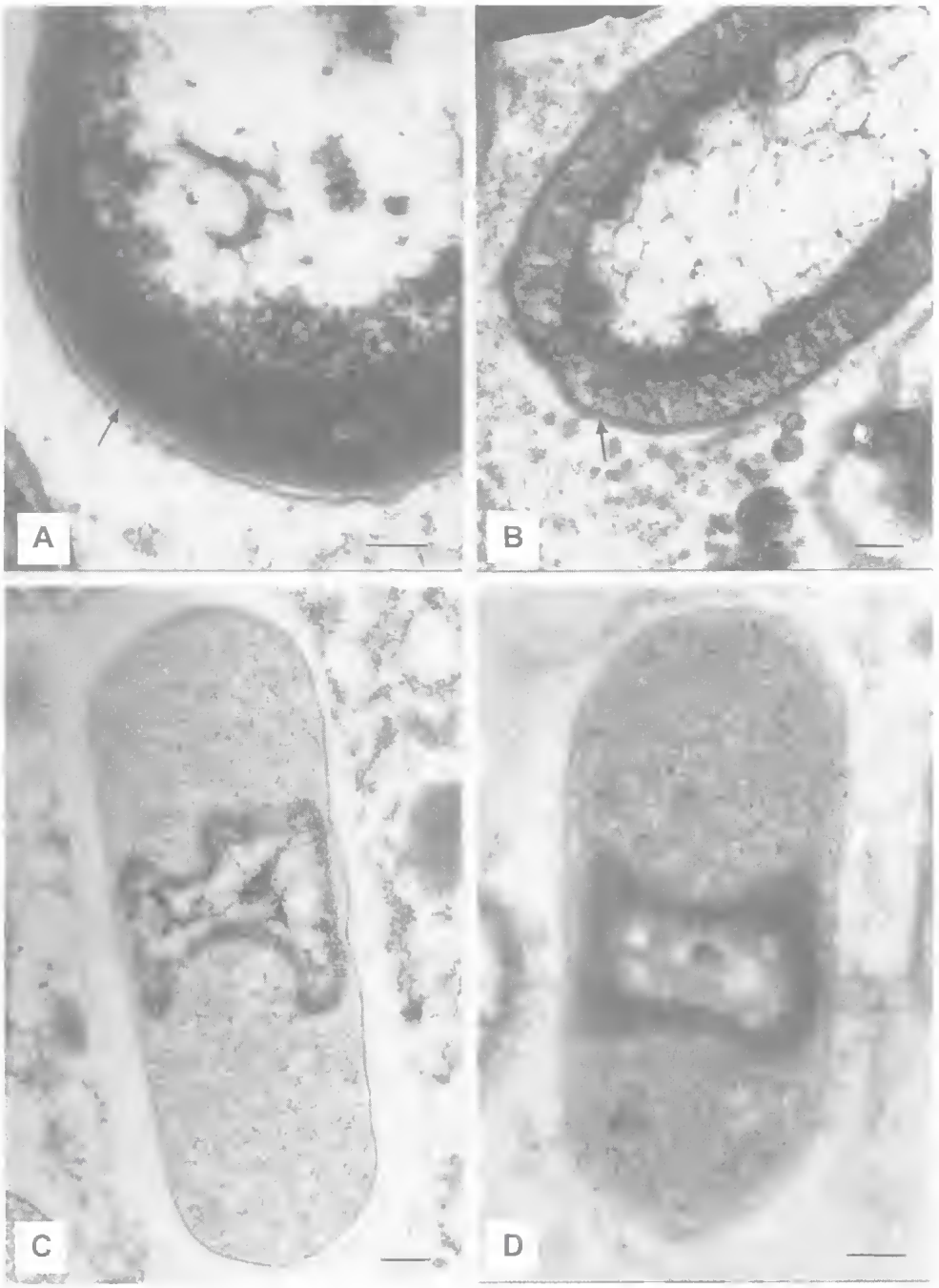

FIG. 3. A-B, Electron micrographs of thin-sectioned Morphotypes 2 and 3. A, Morphotype 2 cell (same cell as shown in Fig. 1C) showing a clear cytoplasmic membrane (arrow) adjacent to the cell wall and external to and widely separated by electron-dense cytoplasm from the nuclear body membrane. (Scale bar 100nm). B, Morphotype 3 showing a cytoplasmic membrane (arrow) closely appressed to the cell wall. (Scale bar 100nm). C, Electron micrograph of thin-sectioned nucleated symbiont Morphotype 1 from *Jaspis stellifera* showing labelling of DNA only within nuclear body, via immunogold detection of mouse monoclonal antibody against single stranded and double stranded DNA (10nm colloidal gold particles). (Scale bar 200nm). D, Electron micrograph of thin-sectioned nucleated symbiont Morphotype 1 from *Jaspis stellifera* showing location of intracellular RNA via labelling with RNase-gold. Note the absence of labelling over the central nucleoid. (Scale bar 200nm).

to be confined to the membrane-bounded internal body, and that all the cell DNA was found outside that body (Fig. 13). Although there is a reverse topology to the compartmentation of DNA found in the other symbiont morphotypes, it would appear that the cell's DNA is still restricted to a separate compartment within the cell as also occurs in the other Morphotypes, via a different compartment. The inner compartment of Morphotype 6 appears superficially to be similar structurally to the nuclear body in the other morphotypes, in the sense of being a single-membrane-bounded inner compartment, but in this case is devoid of DNA.

In their possession of a membrane-bounded nucleoid, the internal organisation of the sponge symbiont Morphotypes 1-5 described here contrasts with that known for most members of Domains Bacteria and Archaea. However, it is most similar to that found previously in planctomycete species of the genus *Pirellula*, where there is enclosure of a major nucleoid-containing cell compartment, the pirellulosome, by a single membrane dividing the cell interior into two regions and where a zone of electron-dense particles around the nucleoid occurs within the pirellulosome (Lindsay et al., 1997). Also relevant may be the double membrane-bounded nucleoid compartment found in another planctomycete, *Gemmata obscuriglobus* (Fuerst & Webb, 1991). In contrast to the cell structure in *Pirellula* species, the sponge symbionts described here do not display any polar differentiation or 'polar cap'. In natural habitat samples, the only bacterial cells appearing to show any similar ultrastructure to those described here are 0.3-0.5μm diameter cells from soil, described with an internal membrane surrounding the nuclear material (Bae & Casida, 1973).

There are also significant similarities, concerning cell shape and nuclear body shape during division, between the symbionts observed here and cells of *Cenarchaeum symbiosum*, a symbiont of the sponge *Axinella* sp. from the Californian coast of the Pacific Ocean determined by *in situ* hybridisation with oligonucleotide probes to be a member of the kingdom Crenarcheota of Domain Archaea (Preston et al., 1996). If the sponge symbionts exhibiting membrane-bounded nucleoids that we have observed prove to be related closely to *C. symbiosum*, this would be highly significant from an evolutionary perspective. This is because cell organisation in Domain Archaea is thought to be exclusively prokaryotic, even though there are many molecular and phylogenetic similarities with eukaryotes and Domain Eucarya (Keeling et al., 1995), and because a membrane-bounded nucleus would have been demonstrated in all three Domains of Life, suggesting its possible status as an ancestral character of the last common ancestor of the three Domains retained only in some lineages of contemporary organisms. *Cenarchaeum symbiosum* has been determined by both 16S rRNA sequencing and DNA polymerase sequencing (Preston et al, 1996; Schleper et al., 1997) to be a member of the Kingdom Crenarcheota within the Domain Archaea, and it may be relevant to the possible occurrence of nucleated cell organisation in sponge symbionts that a crenarcheote origin for the eukaryotes, or at least a crenarcheote/eukaryote clade, has been suggested from phylogenetic analyses based on amino acid sequences from the highly conserved duplicated genes for protein synthesis elongation factors, EF-Tu and EF-G (Baldauf et al., 1996).

Possible identities for the sponge symbionts with membrane-bounded nucleoids include those of a crenarcheotal Archaea member, a planctomycete member of the Bacteria, or a member of the Eucarya. If the latter, however, it must be a mitochondrion-less representative and one which has lost one membrane of the nuclear envelope. It seems most probable that at least some of the symbionts with a membrane-bounded nucleoid are members of the Archaea, since the cell wall in Morphotypes 1, 3 and 5 exhibit subunit structure consistent with an S-layer wall, the most common wall type in the Archaea (König, 1994). The fluorescent probe-labelled symbionts in *Axinella mexicana* identified by Preston et al. (1996) are non-thermophilic members of the *Crenarcheota* within the Domain Archaea, and these show DNA-containing regions with similar morphology via fluorescence microscopy to those seen in the relevant symbionts studied here by electron microscopy. Archaeal nucleoids in the hyperthermophilic crenarcheotes *Sulfolobus acidocaldarius* and *Pyrodictium abyssi* appear to be naked rather than membrane-bounded (Bohrmann et al., 1994; Rieger et al., 1995), but the mesophilic or even psychrophilic crenarcheotes, which have not been cultured or examined via electron microscopy, may well possess different internal structure from those hyperthermophilic representatives of the same Kingdom. Intracellular lamellar membranes

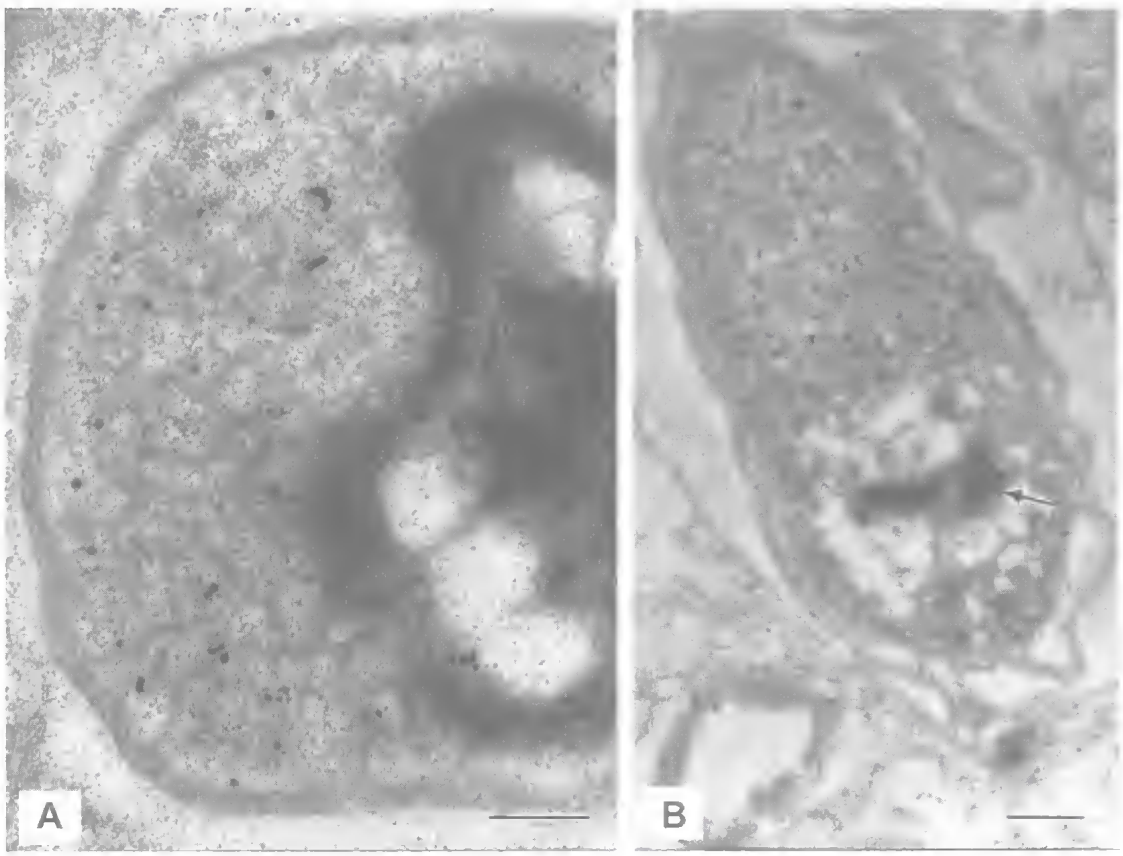

FIG. 4. A, Electron micrograph of portion of a thin-sectioned nucleated symbiont Morphotype 1 from *Jaspis stellifera* which has been double-labelled for both DNA and RNA via use of different sizes of gold particle (10nm for RNase gold and 5nm for anti-DNA antibody labelling via goat anti-mouse IgM Ab conjugated to colloidal gold). (Scale bar 100nm). B, Electron micrograph of thin-sectioned Morphotype 6 symbiont from *Jaspis stellifera* labelled using RNase-gold (large 10nm gold particles) and anti-ss- and ds-DNA antibody immunogold (small dot-like 5nm gold particles) showing the occurrence of RNA but not DNA within the internal membrane-bounded body and the exclusive occurrence of DNA associated with fibrillar nucleoid (arrow) outside the inner membrane-bounded body. (Scale bar 200nm).

found in Type 1 methanotroph-like Bacterial symbionts of deep-sea carnivorous sponges in methane-rich waters (Vacelet et al., 1996) do not appear to be similar to the membranes enclosing the nucleoids described above, which do not display membrane over-folding or multiple layering. Possible Domain membership of the symbionts can be resolved by direct probing of cells in sections using probes specific for 16S rRNA of specific Domains, or via cloning of PCR-amplified 16S rRNA genes from the symbiont community combined with hybridisation of sectioned or whole cells with probes designed from clone sequences. It can be predicted that the symbiont Morphotypes 1, 3 and 5 with regular subunit walls should be found by such methods to be members of the Kingdom Crenarcheota within the Domain Archaea.

CONCLUSIONS

At least 6 morphotypes of bacteria-like symbionts in the mesohyl of the sponge genera *Jaspis*, *Pseudoceratina*, *Axinyssa*, found in the waters of Heron Island, Great Barrier Reef, possess membrane-bounded nuclear regions. In at least 2 of these morphotypes, cell walls composed of subunits are present, consistent with membership of the Archaeal Domain. In this

context it is of great relevance and interest that members of the Domain Archaea belonging to the kingdom Crenarcheota have been found by direct gene probing using *in situ* fluorescent oligonucleotide hybridisation of whole cells to be present in sponges within genus *Axinella* (Preston et al., 1996). These associates or symbionts of this sponge have been referred to as *Cenarchaeum symbiosum*, and phylogenetic analysis using sequences from at least two genes from this species have confirmed membership of the Crenarcheota within the Domain Archaea (Preston et al., 1996; Schleper et al., 1997). In all but Morphotype 6, the blebbed cell wall morphotype, all the cell DNA is in a nuclear body bounded by a single membrane. The extranuclear cytoplasm possesses most of the cell RNA (i.e., it does not appear to be periplasm but true cytoplasm).

The diversity of morphotypes which differ in cell wall type, cell shape, cytoplasm texture and cell compartment type, yet all share compartmentalisation of the cell into two compartments, a nucleoid-containing and a non-nucleoid-containing one, suggests either that somewhat phylogenetically diverse organisms are perhaps phylogenetically related to a common ancestor with a similar form of compartmentalisation which was retained in otherwise structurally diverse descendants, or alternatively that some environmental factor in the sponge tissue selects for or induces nuclear compartmentalisation. It is also possible that nucleated organisms, or organisms with correlated cell wall structure, are selected for by a sponge tissue factor such as a compound with antibiotic activity, for example, a compound inhibiting enzymes involved in DNA synthesis or supercoiling as performed in cells with prokaryote structure and naked chromosomal DNA or one inhibiting synthesis of the peptidoglycan cell wall polymer found in most members of the Domain Bacteria. Classical prokaryotic Bacteria with peptidoglycan walls and naked cytoplasmic DNA may not compete efficiently with nucleated peptidoglycan-less organisms of whatever Domain.

Aspects of these results are significant to our understanding of cell organisation and are fundamental to biology in general. Our results from sponge symbionts may constitute a challenge to the major structural classification of cell types based on cell organisation - that of the prokaryote and eukaryote- since at least some otherwise bacteria-like cell types appear to contain membrane-bounded DNA in a manner analogous to eukaryote cell nuclei. This extends the challenge to that classification first revealed by the discovery of double- and single-membrane bounded nuclear bodies in the planctomycete members of the Division Bacteria. If the Archaeal nature of some of the sponge symbiont morphotypes with nuclear regions can be demonstrated by molecular sequence-based methods, then membrane-bounded nuclei may well be shown to occur at least rarely within members of all three Domains of life, Bacteria, Archaea and Eucarya, a finding which would be of fundamental significance to our understanding of how eukaryote cells may have evolved their initial defining structure. Insights from study of sponge biology may thus yet again contribute to our fundamental understanding of cell biology and evolution.

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